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CYTOLOGY OF HELVELLA
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Contributions to the Cytology of *Helvella crispa*, Fries.

BY

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With Plates XVIII and XIX.

HELVELLA CRISPA, Fries, is a pale buff Ascomycete with a stalked ascophore, the head of which is much convoluted. Material was collected in the autumn of 1909 and was fixed in Flemming's fluid, the strong solution diluted with an equal quantity of water being most satisfactory.

Sections were cut from $3-8\mu$ in thickness, and were stained with Flemming's triple stain, or with Heidenhain's iron-haematoxylin, and in the latter case either erythrosin or *lichtgrün* was used as a counter-stain.

The research was carried out at Birkbeck College, and I wish to express my thanks to Dr. Fraser, at whose suggestion the work was undertaken, for valuable help and advice throughout its progress.

VEGETATIVE TISSUE.

The hypothecium is made up of a number of septate hyphae forming a loose tangle, the cells of which contain fairly abundant protoplasm and one, two, or several nuclei each. These nuclei are small; the chromatin appears to be scattered in small granules round the periphery without any definite reticular arrangement.

Certain of these nuclei were observed to fuse in pairs (Pl. XVIII, Figs. 1, 2), but no evidence of migration such as has been recorded in *Humaria* (7) and in *Nephrodium* (6) was obtained. In most cases the cells of the paraphyses are binucleate, while those of the fertile hyphae have a larger number of nuclei, but no exact differentiation can be traced between the two.

FORMATION OF THE ASCUS.

The hyphae which are to give rise to the asci are generally larger than the paraphyses, and have scantier and less granular contents. Their nuclei resemble in size and appearance the fusion nuclei of the hypothecium.

A certain amount of evidence was obtained as to the mitoses in both

the vegetative and the fertile hyphae (Figs. 3, 4, 5, 6). The former show structures which have every appearance of spindles, with two chromosomes (Figs. 3, 4); the latter have four chromosomes (Fig. 5), and at a later stage eight are visible (Fig. 6). The nuclei are, however, so minute that it would be unwise to attach any great importance to these phenomena. It is conceivable that the 'spindles' are only a fibrillar arrangement of the cytoplasm, and the 'chromosomes' nothing more than some of the granules which are abundant in the hypothecial tissue. It is difficult to account for the occurrence of a bent hypha with two spindles, each bearing four chromosomes (Fig. 5), on the basis of coincidence, and it seems desirable to give the evidence for what it is worth, without accentuating its theoretical importance.

The ascogenous hypha bends over to form the characteristic crozier, and two nuclei pass into the bent portion; these divide simultaneously, and eventually a terminal uninucleate and a penultimate binucleate cell are cut off (Fig. 7). There is some evidence to show that the nucleolus of each nucleus now buds off a portion of itself, which is extruded at one end of the nucleus and is very conspicuous at this stage (Fig. 8).

The two nuclei of the penultimate cell fuse, and the nucleolar granules remain one at either pole of the resulting nucleus (Fig. 8). A projection is formed from the penultimate cell, and into this the large double nucleus passes; it grows rapidly, pushing up among the paraphyses, and forms the young ascus. The terminal cell often fuses with the stalk cell, their nuclei unite, and a second ascus is formed (Figs. 9, 10).

MEIOSIS.

The nucleus of the young ascus presents a characteristic double appearance. It is oval in shape, and at either pole is visible the granule which was extruded after budding off from the nucleolus. Shortly after fusion the chromatin contracts into two close masses, one at either end, and the granules, which have hitherto stained with nucleolar dyes, gradually become impregnated with chromatin (Fig. 12).

As contraction passes off the chromatic granules are expelled from the nucleus (Fig. 13), forming deeply staining pear-shaped bodies which may be seen traversing the nuclear membrane (Figs. 14, 15). In nearly all cases the original granules appear to be extruded, but there are also smaller chromatin bodies which are budded off directly from the chromatic substance of the nucleus. The larger bodies are evidently expelled with some force, leaving behind them a clear track in the cytoplasm (Fig. 14). At a somewhat later stage secondary buds may be formed from the nucleolus (Fig. 16), but their further history was not traced. The extruded bodies gradually degenerate and once more take up nucleolar stains, until finally they are visible only as refractive granules, which at this stage adhere closely to the nuclear membrane (Fig. 20).

As the nucleus passes out of contraction the thread is gradually unwound from either pole (Fig. 13), and in the spireme it is impossible to distinguish the two portions. The thread is distributed over the whole of the nuclear area, and indications of a longitudinal duplication become visible (Figs. 17, 18). The thread is very granular, and on the appearance of the duplication the granules are observed to lie in pairs (Fig. 18). Eventually the thread appears double throughout its length (Fig. 19).

The second contraction is not very marked; a certain amount of aggregation of the nuclear thread towards one side of the nucleus takes place, but it is rather a change in position than in length (Fig. 20). Very little actual contraction of the thread occurs, but the longitudinal fission is temporarily obliterated. Soon the thread loosens out again, the split reappears, and the thread is arranged in irregular loops, in each of which the split is often very evident (Fig. 21).

The spireme next breaks up to form four gemini, the limbs of which still show the longitudinal fission (Fig. 22). The limbs may be twisted together or remain divergent, and the chromosomes gradually contract into the typical heterotype forms, a figure 8 and an incompletely closed 8 being the commonest (Fig. 23). The tendency is, however, for the chromosomes to remain long and thin while they are free in the nuclear area. The final contraction occurs at about the time when the chromosomes pass on to the spindle (Figs. 24, 25, 26).

As the chromosomes begin to contract the nuclear membrane becomes less distinct, although the nuclear area is clearly evident throughout the division. The astral rays are quite distinct and spread round the nuclear area and out into the dense cytoplasm surrounding it (Figs. 24, 27).

The mature chromosomes are typically V-shaped, and in most the fission is obliterated (Fig. 26), but in some it is indicated by a cleft in each limb (Fig. 25). The limbs separate from each other so that each bivalent chromosome is divided transversely, and at this stage some of the daughter chromosomes are V-shaped, owing to the reappearance of the split (Fig. 27). As they pass towards the poles the chromosomes become much elongated (Fig. 28), contracting again as they reach the poles (Fig. 29).

On the reconstitution of the daughter nuclei distinct chromosomes are no longer visible, the whole chromatic substance forming a network (Fig. 30).

On the spindle of the second division four chromosomes reappear, each of which is V-shaped by incomplete fission. In most cases they are attached at the apex of the V, and here the final separation of the limbs occurs (Pl. XIX, Figs. 31, 32). The chromosomes may elongate during their passage to the poles, or they may remain as more or less rounded bodies.

The resulting four nuclei enter upon a short resting stage.

THE THIRD MITOSIS.

The prophases of the third division appear to be passed through very rapidly; the nuclei increase very little in size during the resting stage and are very small at the time of the third division. The spindles, however, are exceedingly clear, and upon them reappear four chromosomes, at first corresponding in shape with those of the second division, but later becoming elongated and much curved or bent.

The chromosomes are at first closely grouped at the equator (Fig. 33), but at a slightly later stage they separate, two passing towards each pole (Fig. 34). Indications of two bent chromosomes at each pole were obtained (Fig. 35), and confirmation of this interpretation is afforded by the mitosis in the spore which shows two chromosomes passing to each pole (Fig. 39). Although instances in which the chromosome number is visible in the third telophase and in the spore or hypothecium are infrequent, yet the sum of the evidence from all these constitutes a strong argument.

The third mitosis is therefore a typical brachymeiosis.

SPORE FORMATION.

Towards the end of the third division the astral fibres are very obvious (Fig. 36), and on the break-down of the spindle each nucleus develops a beak from which radiations proceed (Fig. 37). These rays completely delimit the spore, sweeping round the nucleus until they meet at the opposite pole (Fig. 38). As the spore wall is formed the nuclear beak elongates and finally becomes detached from the wall.

The nucleus then rounds itself up and the beak disappears. The spore nucleus now divides mitotically to form eight secondary nuclei (Fig. 39). Although this is the normal method of spore formation, there very frequently occurs an abnormality whereby the eight nuclei, instead of forming spores, divide again. Asci thus formed, containing sixteen to thirty-two free nuclei, are of fairly common occurrence (Fig. 41).

ABNORMALITIES.

Abnormal hyphae were observed in which the nuclei of the penultimate cell had divided before fusion, and a second ascogenous cell was cut off (Fig. 40). Proliferation of the penultimate cell to form a second and even a third hook also occurs (Fig. 11). Occasionally the nuclei of the ascogenous cell fail to fuse, but pass together into the young ascus, and, at the stage observed, each was undergoing contraction and giving off a chromatin body (Fig. 42).

MORPHOLOGY.

Our knowledge of the developmental stages of the Helvellineae was until recently restricted to the Geoglossaceae, and was due in the main to the work of Dittrich (4) on *Leotia* and *Mitrula*. He also studied *Helvella*,

but had little success, owing to the difficulty experienced in obtaining early stages. His work has recently been supplemented by McCubbin (11), who has described the morphology and development of the ascocarp of *Helvella elastica*, a form with which *Helvella crispa* has certain points in common.

In *H. elastica* there is a very marked difference between the fertile and vegetative hyphae, the former containing several nuclei in each cell, and the latter only two. In *H. crispa*, although there is generally a difference in the size of the hyphae, the number of nuclei is so variable that no definite separation can be based upon it.

Proliferation of the penultimate cell to form another hook, a phenomenon previously observed in *Humaria rutilans* (7), is described as common in *Helvella elastica*, and occurs, though less frequently, in *H. crispa*, as does anastomosis of the terminal and stalk cells. An abnormal hypha in which the penultimate cell has divided is of interest as the only instance observed in this species of an occurrence which appears to be fairly common in *H. elastica*.

SEXUALITY.

Helvella crispa is another example of the disappearance of normal fertilization and its replacement by the fusion of apparently undifferentiated nuclei. In this respect it is entirely comparable with *Humaria rutilans* (7). The second fusion occurs in the ascogenous hypha, and, as in the various forms already described (7, 8, 9), a double reduction takes place in the ascus.

DEVELOPMENT OF THE ASCUS.

Harper, in 1905 (10), describes the formation and further development of the ascus in *Phyllactinia* and *Erysiphe*. In these the ascogenous hypha does not bend over, but the same division and fusion of nuclei occurs as in the more usual hooked arrangement. In *Phyllactinia* there is no change in the chromosome number throughout the life-history, the chromosomes themselves fusing in pairs at nuclear fusion, so that each becomes quadrivalent.

In *Humaria rutilans* (7) no sexual organs are developed, but fusion of vegetative nuclei in pairs occurs. The usual second fusion takes place in the formation of the ascus and is followed by a double reduction. The first two divisions in the ascus are meiotic, but the third is of a simpler type, in which reduction is accomplished without any apparent pairing of the chromosomes. Similar processes have been described by Fraser and Welsford for *Otidea aurantia* and *Peziza vesiculosa* (8). In 1909 Fraser and Brooks (9) described the cytology of the ascus in *Humaria granulata*, *Lachnea stercorea*, and *Ascobolus furfuraceus*, in all of which there is a pseudapogamous fusion in the ascogonium and a subsequent fusion in the ascus. In each case the double fusion is followed by a double reduction in

the ascus, the first two divisions constituting a typical meiosis, and the third brachymeiosis of the type described for *H. rutilans*.

These results have been questioned by Guillermond, who doubts the existence of brachymeiosis. The numerical change in the third division has, however, been recorded, not only by those who accept the occurrence of two successive fusions, but by an observer so little predisposed in its favour as Dangeard (2), who considers that the fusion in the ascus alone takes place.

The essential phenomena in *Helvella* show a strong resemblance to those in *Humaria rutilans*. In both the normal sexual process is replaced by a fusion of vegetative nuclei, and in both meiosis and brachymeiosis occur in the ascus.

There are, however, differences which are of some interest. In *Humaria* the meiotic phase is initiated before the second fusion, and in the resulting nucleus the component nuclei cannot be distinguished. *Helvella* exhibits a less complete fusion, for the two nuclei in the penultimate cell of the ascogenous hypha show no sign of the coming meiosis, unless the casting out of a part of the nucleolus, the basis of the chromatin body, be regarded as a part of the process.

The association of nuclei in the asexual fusion in *Helvella* is less intimate than in any form yet described. The fusion is at first merely a disappearance of the boundary between two nuclear areas, and during the initial stages of meiosis there is no mingling of the chromatin. The first contraction takes place independently in the two masses of chromatin, and it is not until the formation of the spireme that the chromatin threads of the original nuclei cease to be distinct.

It may be possible to correlate this state of affairs with the fact that no pairing of the chromosomes takes place in brachymeiosis.

In *Humaria rutilans* pairing of the brachymeiotic chromosomes is also absent. In this case, although the chromatin masses do not as a rule remain obviously distinct, the first contraction is passed through before fusion, a fact which seems to indicate that the fusion itself is incomplete. Instances were also observed in *Humaria* in which the chromatin was visible in two separate masses in the post-synaptic stage.

There are four times the post-meiotic number of chromosomes in the definitive nucleus of the ascus, but as the first division is heterotype the chromosomes are paired before they appear on the spindle, and the quadruple number is therefore never seen.

MEIOSIS.

The details of meiosis conform to the method described by Farmer and Moore in 1905 (5). Two contractions occur, and while there is no evidence whatever of an approximation of spiremes, all the observed facts fall readily

into line with the process as described by Farmer and Moore and by subsequent workers for a great number of plants.

On any supposition other than the reappearance of a previous fission, the cleft ends of the chromosomes on the first spindle must be recognized as the manifestation of an entirely new development.

A comparison of the chromosomes on the heterotype spindle before and after the separation of the limbs leaves little doubt that transverse separation has occurred. On this view the heterotype division is to be regarded as interpolated into the stages of an ordinary karyokinesis, and the reappearance of the split is therefore to be expected.

The third or brachymeiotic division differs from meiosis in its greater simplicity and brevity. In *Helvella* no contraction takes place, and the process is very possibly to be regarded as merely the separation of the nuclei which fused in the ascogenous hypha.

The occurrence of brachymeiosis has been questioned by Strasburger, who accepts Claussen's (1) view that there is only one fusion, that in the ascus, and that this is the completion of a sexual process between two nuclei which have been long associated. Such a theory necessarily precludes the recognition of a double reduction in the ascus. The evidence concerning the absence of a previous fusion is, however, only negative, and so many cases have been recorded among the Ascomycetes of a sexual followed by an asexual fusion, as to make it evident that this state of things is normal.

Such forms as *Helvella*, with an apogamous and an asexual fusion, exhibit a readily comprehensible reduction from the typical sexual process. Each new instance of two successive fusions forms a further corroboration of brachymeiosis.

THE 'CHROMATIN BODIES'.

The ejection of chromatic substance from the nucleus during the early stages of meiosis was described by Digby (3) in 1909 for *Galtonia candicans*. In *Galtonia* the bodies arise either as nucleolar buds which are later infiltrated with chromatin, or as outgrowths from the nuclear framework itself. They remain attached to the nucleus by long fine threads which persist even after the bodies have passed into a neighbouring cell. This phenomenon occurs most abundantly during synapsis, but frequently also in the earlier stages.

In *Helvella* the course of events is strikingly similar. The chromatin bodies may arise either from the nucleolus or nuclear framework, and in the former case they become gradually impregnated with chromatin. They are then forcibly ejected from the nucleus, often clearing a path in the cytoplasm, a fact which is of some interest, as the *Galtonia* bodies have usually a clear space round them. The somewhat pear-shaped body is

drawn out behind into a fine thread, by means of which it remains attached to the nucleus for a considerable time, but never at a great distance from it.

A relationship has been suggested between such bodies and the chromidia cast out during Gametogenesis in the lower animals, but it is at present still uncertain whether any special significance with regard to meiosis can be attached to the process. If the extrusion of a part of the chromatin could be shown to be a normal accompaniment of meiosis and to form an integral part of the process, it might perhaps be held that not only the qualitative halving of the chromosomes, but the loss of some constituent of the nucleus was necessary to the development of sexual cells. In *Helvella*, however, meiosis takes place many cell generations before any fusion, and sexual cells are never differentiated. In such a case it is possible that the chromatin bodies are no more than masses of nutritive substance, for which the nucleus has no further use. Against such a view is to be set the fact that the process is as well defined and elaborate in the Ascomycete as in the Angiosperm, and such a phenomenon occurring with striking similarity in widely separated groups is suggestive of some deep-seated analogy.

In drawing conclusions from these facts, it must, however, be remembered that *Helvella* is certainly a reduced form, and the expulsion of the bodies may be only a vestigial phenomenon. Even if it occurred in the ancestral type, which may reasonably be assumed to have possessed sexual organs as well developed as those of *Pyronema*, it is difficult to see what necessary connexion existed between the extrusion of chromatin masses and meiosis.

SUMMARY.

1. There are no sexual organs in *Helvella crispa*, but fusion of nuclei in pairs occurs in certain hyphae of the hypothecium, and these cells produce the ascogenous hyphae.
2. There is evidence that mitoses in the vegetative and ascogenous hyphae show respectively two and four chromosomes.
3. A second fusion occurs in the formation of the ascus, but the chromatin of the two nuclei remains distinct until the spore stage.
4. The first and second divisions in the ascus constitute a meiotic phase of the type described by Farmer and Moore.
5. During the early stages of meiosis chromatin bodies are extruded from the nucleus.
6. In the first two divisions four chromosomes appear on the spindle.
7. The third division is brachymeiotic; in the prophase four chromosomes appear and two pass to each pole.

8. The spores are outlined by radiations from the centrosome, and when mature contain eight nuclei; the divisions in the spore show two chromosomes.

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EXPLANATION OF PLATES XVIII AND XIX.

Illustrating Miss D. Carruthers' paper on the Cytology of *Helvella crispa*.

All figures were drawn with a Zeiss apochromatic immersion lens, apert. 1.30 and compens. oc. 12 (magnification about 2000), with the exception of Fig. 41, for which compens. oc. 6 was used.

PLATE XVIII.

- Fig. 1. Apogamous fusions in the hypothecium.
- Fig. 2. The same, later stage.
- Fig. 3. Mitosis in vegetative hypha.
- Fig. 4. Telophase of same.
- Fig. 5. Prophase in ascogenous hypha, showing four chromosomes.
- Fig. 6. Metaphase in fertile hypha, showing eight chromosomes.
- Fig. 7. Crozier with two nuclei in penultimate cell.
- Fig. 8. Fusion in ascus.
- Fig. 9. Connexion between stalk and terminal cell.
- Fig. 10. Nucleus migrating from terminal to stalk cell.
- Fig. 11. Formation of successive croziers.

FIRST DIVISION.

Fig. 12. Definitive nucleus of ascus, with chromatin of two nuclei undergoing first contraction separately.

Fig. 13. Emission of chromatin body, spireme stage.

Fig. 14. Same, later stage.

Fig. 15. Same, showing thread passing through nuclear membrane.

Fig. 16. Secondary buds from nucleolus.

Fig. 17. Spireme with longitudinal fission.

Fig. 18. Ditto.

Fig. 19. The fission complete.

Fig. 20. Second contraction.

Fig. 21. Nucleus passing out of contraction, the fission once more visible in the loops

Fig. 22. Spireme broken up into chromosomes.

Fig. 23. Immature chromosomes.

Fig. 24. Chromosomes on spindle, centrosome and aster visible.

Figs. 25, 26. Chromosomes on spindle.

Fig. 27. Metaphase with eight daughter chromosomes.

Fig. 28. Chromosomes passing to poles.

Fig. 29. Telophase.

Fig. 30. Reconstitution of daughter nuclei.

PLATE XIX.

SECOND DIVISION.

Fig. 31. Chromosomes breaking apart.

Fig. 32. Spindle with eight daughter chromosomes.

THIRD DIVISION.

Fig. 33. Prophase of third division.

Fig. 34. Metaphase, two chromosomes passing to each pole.

Fig. 35. Telophase.

Fig. 36. Late telophase with astral rays.

Fig. 37. Formation of nuclear beaks.

Fig. 38. Spore formation.

Fig. 39. Mitosis in spore.

Fig. 40. Proliferation of terminal cell of crozier.

Fig. 41. Secondary nuclei formed by division without spore formation.

Fig. 42. Two nuclei in ascus, which have failed to fuse.



1a.



3.



5.



1b.



4.



2.



6.



7.



8.



11.



10.



9.



12.



13.



14.



15.



16.





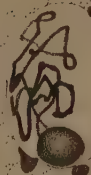
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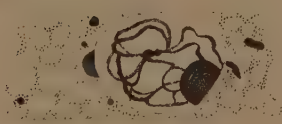
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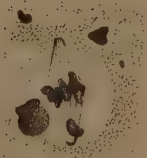
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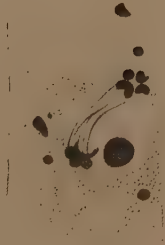
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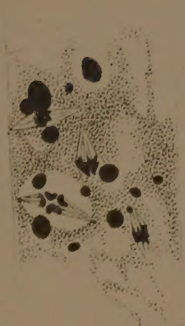
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Huth, lith et imp.

